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Microbe–mineral interactions: The impact of surface attachment on mineral weathering and element selectivity by microorganisms

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ABSTRACT

One of the major gaps within the field of biogeochemistry is the lack of a detailed and deep understanding of the mechanism behind the microbial inducement of mineral dissolution. The association of microorganisms with the mineral surfaces is an important issue for understanding processes like mineral weathering, biomineralization, bioremediation and biofouling. The present study aims to investigate the performance of attached and unattached soil fungal and bacterial species in biotite weathering and in the selectivity of elements from biotite. Sterilized microplate devices were filled with biotite (>2 mm) followed by an iron limited liquid growth medium and were inoculated separately with six different microbial species isolated from podzol soil: *Erwinia amylovora*, *Pseudomonas stutzeri*, *Pseudomonas mendocina*, *Streptomyces pilosus*, *Neurospora crassa* and *Penicillium melinii*. The experiment was designed in two set-ups: 1) attached form, in which the microorganisms were inoculated directly to the biotite surface, and 2) unattached form, in which 0.4 μm PET track etched devices were used to separate the microbial cells from the biotite surface.

Our findings indicate that the surface attached microorganisms led to a greater dissolution of elements from biotite than the unattached microorganisms that was evidenced by 1) higher dissolution of Fe, Al and Si, 2) greater decrease in pH of the liquid growth medium, and 3) relatively higher production of siderophores. Furthermore, there was no significant difference in the capability of element selectivity between the attached and unattached microbial forms. The biotite dissolution was promoted initially by complexation processes and later by acidification processes for most of the attached and unattached microorganisms. Thus, we conclude that despite the mineral dissolution induced by microbial attachment on the mineral surface, the element composition of the biotite and nutritional need of the microorganisms were the main factors affecting the element selectivity.

1. Introduction

More specific details regarding the mechanism behind microbe–mineral interactions are needed for a deeper understanding of a variety of environmental issues such as weathering reactions involved in nutrient cycling, neutralization of acidic rain and long term drawdown of atmospheric CO_2 (Drever and Hurcomb, 1986; Berner and Berner, 1997; Huntington et al., 2000; Miot et al., 2014). Although most previous studies have focused on weathering as a mineral–solution interaction process, a few studies have emphasized the potential importance of local microbial effects on mineral dissolution caused by surface attachment of the microorganisms (Banfield et al., 1999; Rosling et al., 2004; Buss, 2006).

Microbial attachment on mineral surfaces is a rapid process and is extensive in both aqueous and soil environments (Brisou, 1995; Jones and Bennett, 2014). Attachment is mostly dependent on the great tendency of organic compounds to adsorb to mineral surfaces and to form a surface layer that attracts the attachment of microorganisms looking for an easily available nutrient source (Little et al., 1997). As a result, unattached (suspended in associated solution) microorganisms in natural environments represent only 0.1 to 1.0% of the total biomass of microorganisms (Madigan et al., 2000). Furthermore, it has been estimated that the number of unattached microorganisms in soil solution is about 10^6 ml^{-1}, while the number of microorganisms attached to soil organic particles is about 10^8 g^{-1} (Mills, 2003). The differences between attached and unattached microbial composition may depend on the selective changes conferred on some communities by attachment to surfaces (Marshall, 1992; Mills, 2003). Attachment of individual microbial cells has been associated with changes in cell physiology (Van Loosdrecht et al., 1990; Lehman et al., 2001); however, the manner of those physiological changes and the factors affecting them are still unclear.

The attached and unattached microorganisms behave differently in their interaction with minerals. Microbial attachment to mineral surfaces leads to the formation of a microenvironment (microbial biofilms containing extra-polysaccharides) that protects the microorganisms

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against environmental stress (Beveridge et al., 1997; Liermann et al., 2000a; Ojeda et al., 2006). One of the benefits of microenvironments is the fact that nutrients can be chelated directly from the surrounding mineral surfaces by certain microorganisms or shared among the surrounding microorganisms (Rogers and Bennett, 2004; Uroz et al., 2009; Ahmed and Holmström, 2014a). Attached microorganisms dissolve minerals by a variety of mechanisms such as increasing the concentration of protons, inducing the formation of mineral surface ion complexes, catalyzing redox reactions and using physical forces (Rogers et al., 1998; Brown et al., 1999; Bennett et al., 2001; Roberts Rogers et al., 2001). The direct attachment between the microorganisms especially fungi and mineral surface enhances the weathering by an early mechanical force by the hyphae that increases the mineral surface area in concert with later chemical weathering processes, which in turn promote further breakdown of the mineral (Bonneville et al., 2009, 2011). Unattached microorganisms, on the other hand, live as individuals or in aggregates in solution where they obtain their element nutrition mainly by forming complexes with dissolved ions and by oxidation–reduction reactions (Madigan et al., 2000).

Due to the importance of silicate minerals in soil formation and as nutritional source in terrestrial ecosystems, the quantification of microbial weathering performance and a detailed understanding of the underlying processes are of particular interest. In addition, there is a lack of knowledge about the importance of attached and unattached microorganisms within mineral weathering mechanisms. Thus the main aim of the present study is to investigate the impact of surface attached and unattached soil microbial species on biotite dissolution and element selectivity from biotite in an attempt to gain a better understanding of the weathering performance of the mineral attached and free living microorganisms in soil environment.

2. Materials and methods

2.1. Experiment set-up

Biotite used in the present experiment was purchased from Wards, Canada. Mineral samples were crushed with ceramic ball mills and was then sieved to collect the >2 mm size fraction. The samples were then washed and sonicated in distilled deionized water at neutral pH to remove ultrafine particles. The element composition of the biotite surface was analyzed using scanning electron microscopy energy dispersive X-ray spectroscopy (SEM–EDX) (JEOL JSM-7000F, Japan). Chemical data showed that the biotite surfaces were made up by SiO_2 39.6%, Al_2O_3 11.04%, FeO 16.50%, Fe_2O_3 1.61%, MgO 14.49%, TiO_2 2.08%, MnO 0.70% and K_2O 9.35%. The mineral samples were sterilized at 170 °C for 1 h under a dry atmosphere before the beginning of the experiment. Previous studies demonstrated that the dissolution kinetics of biotite was not modified by autoclaving (Calvaruso et al., 2006; Balland et al., 2010; Balland-Bolou-Bi et al., 2014).

Four bacterial species (*Erwinia amylovora*, *Pseudomonas stutzeri*, *Pseudomonas mendocina*, *Streptomyces pilosus*) and two fungal species (*Neurospora crassa*, *Penicillium melinii*) were used in the experiments which were isolated from a podzol soil profile in the middle of Sweden (Ahmed and Holmström, in press). The microbial cultures were individually inoculated into 25 ml of growth medium and thereafter incubated on a rotary shaker (200 rpm) for 2–4 days at room temperature. The biomass was harvested by centrifugation at 3000 rpm for 10 min, then washed twice with sterilized MilliQ water and again centrifuged at 3000 rpm for 10 min to collect the clean and sterile microbial cells.

Sterile microplate devices containing six wells (VWR International, Sweden) were used for the mineral dissolution experiments. All microplate wells were filled with 22 mg of biotite followed by addition of 15.5 ml of an iron limited liquid growth medium (glucose 5 g/l, tryptone 17 g/l, NaCl 0.025 g/l, $(NH_4)_2HPO_4$ 0.025 g/l, KH_2PO_4 0.05 g/l). Under these conditions the biotite was the only source of trace elements required for microbial growth. Afterwards, 100 μl of the harvested cells were inoculated separately in each well with two replicas.

Mineral dissolution experiments were designed in two set-ups, 1) attached form, in which the microorganisms inoculated directly to the biotite surface, and 2) unattached form, in which 0.4 μm PET track etched devices (VWR International, Sweden) were used to separate the microbial cells from the biotite surface (Fig. 1). To characterize the weathering of biotite, we collected 3 ml samples from the microplates in intervals, after 4, 7, 10, 13, and 16 days. Uninoculated biotite inserted in the growth medium was used as a control.

2.2. Analysis of solution pH, dissolved elements and siderophore production

The sub-samples of the solutions from the bioweathering experiments were filtered through 0.45 μm millipore filter (Filtropur S, Sarstedt, Germany) and were then centrifuged at 10,000 rpm for 10 min to remove microbial cells before the chemical analysis. Three milliliters of the collected supernatant was used for pH determination and then 2 ml was acidified with HNO_3 and diluted by MilliQ water (final acid concentration 2% v/v) to avoid precipitation of dissolved elements. All the samples were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Varian Vista AX, SPS-5, USA) to determine the concentration of dissolved elements.

For the siderophore determination, the remaining 1 ml supernatant was pre-concentrated by freeze-drying (Scanvac Cool Safe, 100-9 Pro). The remaining yellow brown solid dust was dissolved in 0.5 ml of MilliQ-water after the freeze-drying. Centrifugal ultrafiltration 3000 Da cutoff filters (Nanosep 3K Omega, Pall, Mexico) were used to remove the high molecular mass compounds (>3000 Da). Then the filtrates were used for quantification of the siderophore concentration using high performance liquid chromatography coupled to electrospray ionization mass spectrometry (HPLC-ESI-MS) (Ultimate 3000 RS, Thermo Scientific, USA). The ferric complexes of the hydroxamate siderophores (ferrioxamines (E, B, D, G), coprogens (coprogen, neocoprogen II, Fe-dimerum acid) and ferrichrome) were detected by selected ion monitoring (SIM) of the proton adducts. For measurement protocol and instructions, see Ahmed and Holmström (2014b).

2.3. Statistical analyses

The data were normalized and one/two-way ANOVA for multiple comparisons were used to compare the averages for Fe, Al and Si released from the mineral by the attached and unattached microbial species. The statistical analyses and ternary plots were carried out using XLSTAT (http://www.xlstat.com/en/). The calculations of the saturation index (S.I.) with respect to secondary mineral phases (Fe and Al oxides and clays) were calculated using Visual MINTEQ 3.1.

3. Results

3.1. Dissolution of biotite by attached and unattached microorganisms

3.1.1. pH

All the microbial species lowered the pH of the solution during the 16 days of the experiment. In general the mineral attached microorganisms decreased the pH of the growth medium to a greater extent than the unattached microorganisms (Fig. 2A, B). The attached microorganisms decreased the pH to 2.7–3.7, whereas the unattached microorganisms decreased the pH to 3.6–4.4. On the other hand, there was a variation between the different bacterial and fungal species (Fig. 2A, B). Inoculation of the attached fungi *N. crassa* resulted in a fast pH decrease, which reached pH 2.7 after 16 days, compared with the unattached form that decreased the pH to 3.6. The fungal strain *P. melinii* resulted in a slower pH decrease. It decreased the pH to 3.5 for the attached form and to pH 4.4 for the unattached form after 16 days. Although the bacterial species did not have a remarkable difference in their pH decrease,